REMARKS

Entry of the foregoing and reconsideration of the subject application, as amended, pursuant to and consistent with 37 C.F.R. Section 1.112, and in light of the remarks which follow, are respectfully requested.

An one month petition for extension of time is being filed concurrently with this Amendment.

New claim 22 is supported by paragraph [0032]. New claim 23 is supported by paragraph [0038].

Claims 1, 2, 9, 11-13, 15, and 18-21 have been rejected under 35 U.S.C. Section 103(a) as purportedly being obvious over Hitchcock (U.S. Patent 2,622,976) in view of East German Patent Application Publication DD257379A (hereinafter Bergmann). In addition, claim 10 has been rejected under 35 U.S.C. Section 103(a) as purportedly being obvious over the combination of Hitchcock, Bergmann, and Watanabe et al. (hereinafter Watanabe). Those rejections are respectfully traversed.

The present invention, as embodied in claim 1, concerns a method for reducing pests in an object or area, said method comprising applying to said object or area a pest reducing effective amount of a compound selected from the group consisting of iodoacetic acid, bromoacetic acid, 2-iodoacetamide, 2-bromoacetamide, and mixtures thereof. Claim 10 states that the weeds are selected from the group consisting of *Amaranthus hybridus*, *Echinocloa crus-galli*, *Cyperus rotundus*, and mixtures thereof. Due to the election of species, the compound is bromoacetic acid and the pests are weeds (i.e., *Cyperus rotundus*).

The primary reference Hitchcock discloses a method of controlling the growth of weeds through the pre-emergence treatment of soil with an aqueous solution of chloroacetic acid in an amount of 20 to 40 pounds of chloroacetic acid per acre.

The present invention differs from Hitchcock in that the present invention utilizes bromoacetic acid (as elected) whereas Hitchcock utilizes chloroacetic acid.

Since the primary reference Watanabe fails to disclose monobromroacetic acid, the Examiner has been forced to rely upon the secondary reference Bergmann which discloses a synergistic herbicide containing a **combination of** 3,5-dibromo-4-hydroxy benzonitrile (Compound I) or 3,5-diiodo-4-hydroxy benzonitrile (Compound II) **and** (A) monochloroacetic acid (Compound III) or monoiodoacetic acid (Compound IV). The object of Bergmann was the desiccation of a crop plant (i.e., potato haulm (stems)) and reduction of tuber infection caused by the fungus *Phytophthora infestans*.

The tertiary reference Watanabe discloses a synergistic herbicidal composition for combating the undesired vegetation of perennial weeds of Cyperaceae (*Cyprus* spp. (sedges)) and Gramineae. The composition contains as active ingredients at least one of the herbicidal compounds having contact acute phytotoxicity (compound A) **and** at least one of the herbicidal compounds having translocated chronic phytotoxicity (compound B). Compound A is described at column 1, lines 53-66 and includes chlorinated aliphatic acids (e.g., monochloroacetic acid). Compound B is a fluoropropionic acid. The preferred quantity by weight for monochloroacetic acid or its salt or amide is 5 to 10 kg/10a (0.11 to 0.44 lb/a). The quantity by weight for compound B is 0.2 to 1.0 kg/10a. In the examples the composition was applied to areas where weeds were growing.

The Examiner has alleged the following (page 4, Office Action; emphasis added):

> ...One of ordinary skill in the art at the time the instant application was filed would have had a reasonable expectation of success in substituting bromoacetic acid in place of chloroacetic acid, because **bromoacetic acid would reasonably be expected to possess similar physicochemical properties to those of chloroacetic acid due to their substantial structural similarities**....

However, it is common knowledge in the field of pesticide/herbicide chemistry that no two compounds, regardless of structure similarity, can be expected to have any or similar efficacy. Highly relevant to this fact is U.S. Patent 6,465,527 (copy attached). Based on the Examiner's allegation above, it would be argued that one of ordinary skill in the art at the time the instant application was filed would have had a reasonable expectation of success in substituting 1,2-diiodoethane in place of 1,2-dibromoethane because 1,2-diiodoethane would reasonably be expected to possess similar physicochemical properties to those of 1,2-dibromoethane due to their substantial structural similarities. However, **in fact**, Table 1 showed that 1,2-dibromoethane was very effective after one hour exposure against the German and American cockroach whereas 1,2-diiodoethane was **not** effective. In addition, Table 2 showed that 1,2-dibromoethane was very effective after 24 hours exposure against the German cockroach whereas 1,2-diiodoethane was **not** effective. This utterly destroys the Examiner's basis for rejecting the claims.

Similar information is provided by the second paragraph under "Results" in the attached article "Leaf Abscission Induced by The Iodide Ion" (Herrett, R.A., et al., Plant Physiology, 3: 358-363 (1961)). Compounds with different halogens were compared as plant defoliants. In table II (page 2), KI is compared to KBr, KCl, and KF. KI had 90% efficacy as a plant defoliant whereas KBr, KCl, and KF demonstrated 0% efficacy, and Herrett stated the following (page 359,

right column): "...Only the iodide ion induced defoliation (table II)...." Yet more evidence that similar halogenated compounds do **not** possess similar properties is found in Table 1 of the attached article "Monofluoroacetic Acid And Related Compounds" (Maynard Chenoweth, Pharmacol. Rev., 1: 383-424 (1949)). Again, this utterly destroys the Examiner's allegation that similar halogenated compounds would reasonably be expected to possess similar physicochemical properties due to their substantial structural similarities.

Furthermore, entries for bromoacetic acid, chloroacetic acid, iodoacetic acid, and fluoroacetic acid from The Merck Index (attached) describes only chloroacetic acic having a known use (i.e., as a herbicide; the sodium salt of fluoroacetic acid was known to be used as a rodenticide). This information regarding chloroacetic acid is also supported by the information (attached) taken from the website http://www.chemicalland21.com/industrialchem/organic/CHLOROACETIC%20ACID.htm and from the website http://www.weblakes.com/toxic/CHLOROACETIC_ACID.HTML which stated the following (emphasis added):

> CHLOROACETIC ACID
> ...Uses
> * Chloroacetic acid is used in the manufacture of cellulose ethers (used mainly for drilling muds, detergents, food, and pharmaceuticals), as a **post-emergence contact herbicide and defoliant**, and in the manufacture of glycine and thioglycolic acid. (2,8)
> * Chloroacetic acid is also used in the manufacture of various dyes, synthetic caffeine, and organic chemicals.

Information (attached) regarding applications of bromoacetic acid taken from the website http://www.chemicalland21.com/industrialchem/organic/BROMOACETIC%20ACID.htm

states the application (use) for bromoacetic acid as "...a chemical intermediate for the manufacturing [of] other compounds and pharmaceuticals." Thus the prior art provides no basis for the allegation that bromoacetic acid would reasonably be expected to possess similar physicochemical properties to those of chloroacetic acid due to their substantial structural similarities.

In addition, it is common knowledge in the field of pesticide/herbicide chemistry that a compound cannot be expected to have any or similar efficacy against *Cyperus* species even if it works on other plant species. This is shown by Hitchcock's own varying efficacy data on different weeds in Table 1 which shows that not all weeds were equally susceptible to a common herbicide. Neither Hitchcock or Bergmann listed or demonstrated any activity of chloroacetic acid against *Cyperus* or *Cyperus* tubers, and Watanabe only demonstrated a mixture and not chloroacetic acid alone against *Cyperus* plants. *Cyperus* has underground tubers which many herbicides will not kill even though they work on other weeds; for example, diuron has activity against broadleaf weeds and grasses yet still does not affect nutsedge (see attached copy of Weed Susceptibility Chart, Agamalian, H., Bell, et al., 2006; available at http://wric.ucdavis.edu/information/weedchart.html). Furthermore, it is well known in the art that no two chemicals, much less a family of chemicals, regardless of structural similarity, can be expected to have the same life-time in the soil, or have the same plant uptake in the xylem or root system of all weeds, or have the same solubility in water.

Also highly relevant to the rejection of the claims is the fact that Bergmann stated the following (page 2 of translation, emphasis added):

> ...The object [of his invention] consists in finding advantageous combinations of **known** herbicide active ingredients for use in modern agrotechnical processes that do not cause damage to the succeeding cultures and reduce the infestation of potato tubers with Phytophthora infestans....

Bergmann then cites only two members of the monohaloacetic acid family, chloroacetic acid and iodoacetic acid, for a good reason: Bergmann specifically stated in his abstract that he only wanted to include "**known** herbicide active ingredients" in his "advantageous combinations." This specifically indicates that there were only two members in the monohaloacetic acid family that were considered "**known** herbicide active ingredients" at the time of Bergmann's invention since Bergmann specifically omits the other three members of the monohaloacetic acid family (i.e., bromoacetic acid, fluoroacetic acid, and astatoacetic acid). Obviously Bergmann was well skilled in the art and would have listed the members of the monohaloacetic acid family that he considered to be "**known** herbicide active ingredients" and pertinent to his objectives.

However, the Examiner has alleged the following (page 4, Office Action; emphasis added):

> ...One of ordinary skill in the art at the time the instant application was filed would have been motivated to substitute bromoacetic acid in place of chloroacetic acid, because **the Bergmann '379 publication reasonably teaches the interchangeability of monohalogenated acetic acids**, which include fluoroacetic acid, chloroacetic acid, bromoacetic acid, and iodoacetic acid, for utilization as a herbicide....

However, contrary to the Examiner's baseless allegation that Bergmann teaches interchangeability among **all** the monohalogenated acetic acids, Bergmann actually teaches only interchangeability among **two** monohalogenated acetic acids (i.e., chloroacetic acid and iodoacetic acid) because those were the only two monohalogenated acetic acids that were disclosed by Bergmann as being "**known** herbicide active ingredients." Bergmann also did not specifically teach using either chloroacetic acid or iodoacetic acid alone so one cannot assume that even the activity of either chloroacetic acid or iodoacetic acid, when used alone, would have interchangeability because one does not know the pure relative contribution of either to the efficacy against weeds in general (much less against *Cyperus* tubers). It should also be noted that Bergmann's **only** reference to the prior art in regard to monohalogenated acetic acids was the citation of United States patent 2,622,976 (Hitchcock) which only teaches only the use of monochloroacetic acid.

Thus Bergmann does not specifically teach or suggest using either chloroacetic acid or iodoacetic acid alone (or any other monohaloacetic acid alone for that matter), nor does he teach or demonstrate their efficacy, as a mixture or alone, against weeds (especially *Cyperus*) since Bergmann demonstrated the use of his composition on **food crops** (i.e., potatoes and tomatoes). Therefore, one cannot assume that either chloroacetic acid or iodoacetic acid alone (or any other monohaloacetic acid alone for that matter) would be effective against weeds (especially *Cyperus*).

Furthermore, Hitchcock teaches the use of chloroacetic acid (not monohaloacetic acids as a family) only for certain specific weeds (Table 1), and even then the weeds were susceptible at varying concentrations of chloroacetic acid. Hitchcock does not teach or demonstrate the use of chloroacetic acid against *Cyperus* or *Cyperus* tubers which are much harder to kill than the common garden variety weeds listed in Hitchcock's Table 1 (see also attached copy of Weed

Susceptibility Chart from UC Davis). One cannot assume that the other members of the monohaloacetic acid family would perform well against a weed tuber (*Cyperus*) when even chloroacetic acid was not used against it.

The Examiner's allegation that one of ordinary skill in the art would have a reasonable expectation of success if bromoacetic acid were interchanged with chloroacetic acid is false as shown above. Furthermore, the Examiner is basing his assumption on the positive results and the material that he took from studying the present patent application and thus is putting forth an assumption based on hindsight which is strictly prohibited (*Grain Processing v. American Maize*, 5 USPQ2d 1788, 1792 (Fed. Cir. 1988)). One has to find a reasonable expectation for the success of bromoacetic acid, alone, especially against *Cyperus* tubers, without using the present application. All three references utilized by the Examiner in this Office Action (Bergmann, Hitchcock, and Watanabe) would definitely be considered to have at least ordinary skill in the art, yet the fact remains that not one of the three listed or demonstrated bromoacetic acid, fluoroacetic acid, or astatoacetic acid (all members of the monohaloacetic acid family) as a herbicide. Nor did any of the three chose to list or demonstrate the use of bromoacetic acid on *Cyperus* or *Cyperus* tubers, especially at the rate of 40-1200 (or 100-400) pounds per acre. Instead all three references chose to use the "**known** herbicide active ingredient" chloroacetic acid. One has to ask the following question: why did the prior art not refer to bromoacetic acid, fluoroacetic acid, or astatoacetic acid if these compounds were "**known** herbicide active ingredients"? If there was a reasonable expectation of success, as alleged by the Examiner, would not at least one of the three (Bergmann, Hitchcock, or Watanabe) have listed bromoacetic acid given that it is said by the Examiner to be obvious to one with ordinary skill in the art?

The claims do not stand or fall together. Claim 22 states that the pest reducing effective amount is 100-400 pounds/acre. None of the references teach or suggest such an application rate. In addition, Claim 23 states that the weeds are tubers of *Cyperus rotundus*. None of the references involve such hard to kill tubers.

Withdrawal of the rejection of the claims under 35 U.S.C. Section 103(a) is respectfully requested in view of the above.

In view of the foregoing, further and favorable action in the form of a Notice of Allowance is believed to be next in order, and such action is earnestly solicited.

Please charge any required fees pertaining to this Amendment to the Deposit Account of the undersigned, No. 50-2134, and credit any overpayment to said Account.

Respectfully submitted,

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Date: 19 April 2007

Leaf Abscission Induced by The Iodide Ion [1]

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Introduction

In the course of biological screening for herbicidal activity in synthetic compounds, various organic iodide complexes had marked defoliating properties when applied to leguminous test plants. Simple iodides such as the salts of alkali metals also caused defoliation. Although this effect has been reported in the patent literature (4, 8), our present investigation was undertaken principally to learn more about some of the factors influencing iodide induced abscission as well as the relationship between this effect and the general problem of foliar abscission.

Materials & Methods

Laboratory reagent grade chemicals were generally employed as the source of the iodide ion. In some instances, however, various organic iodide complexes were synthesized and purified in the laboratory. Concentrations ranging from 0.44 to 7.35 $\times 10^{-2}$ M of the iodide ion in tap water containing 0.05 % by weight of Triton X-155 caused effective defoliation when applied to plant foliage. However, rates at the lower end of this range (0.44 to 2.94 $\times 10^{-2}$ M) induced effective leaf drop and were used in most of the experiments.

The defoliant solutions were sprayed onto test plant foliage using a Beltsville type conveyer belt sprayer equipped with a No. 6502 or No. 6503 standard Tee-jet nozzle. The speed of the conveyer belt was adjusted so that leaves were wet to incipient run-off.

Unless otherwise indicated, the test plants were *Phaseolus vulgaris* var. Tendergreen. Six to eight bean seeds were planted per 4 inch pot in a composted light loam type soil. Uniformity was achieved by thinning to between three and five plants per pot prior to treatment. Treatments were usually applied when the primary leaves were 50 to 60 mm long and prior to the expansion of the first trifolate. This stage was between 10 and 11 days after planting. The percentage defoliation was obtained by counting the number of primary leaves which abscised for each plant and calculating the percentage abscission on the basis of the total number of primary leaves in that pot. The data are averages of between three and five replicate pots which comprised each treatment.

The plants were grown under 16 hours of light either artificially supplied by a combination of fluorescent and incandescent lamps providing approximately 1,000 ft-c at plant level or in the greenhouse with supplementary incandescent lamps to provide the difference between natural day length and a day length of 16 hours.

Results

Indicated in table I are data supporting the point made in the introduction; organic iodide complexes are capable of inducing premature abscission of Tendergreen bean leaves. The data also support the point that simple inorganic iodide salts of alkali metals such as sodium and potassium also cause premature abscission. From field experiments, this phenomenon was demonstrated on field-grown beans.

In order to establish whether or not iodide ion was the only halide capable of causing defoliation, solutions of KI, KBr, KCl, and NaF were sprayed

Table I

Abscission of Leaves of Mature Tendergreen Bean Plants by Various Organic Iodide Complexes & Inorganic Iodide Salts

Compound name	Iodide conc $\times 10^{-2}$ M	% Defoliation
Methylisothiouronium iodide	5.65	80
Dibutyl butylamidophosphate magnesium diiodide	4.47 2.24	73 17
Dibutyl butylamidophosphate zinc diiodide	4.17 2.09	83 80
Dibutyl butylamidophosphate cobaltous diiodide	4.24 2.12	90 93
Potassium iodide	7.35 3.68	97 93
Sodium iodide	8.20 4.10	97 97
Untreated control	...	10

[1] Received revised manuscript Dec. 8, 1961.
[2] Present address: University of California, Davis, Cal.
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Table II

Effect of Various Halogens on Abscission of Primary Leaves of Tendergreen Beans

Halogen salt	Halogen conc ($\times^{-2}$ M)	% Defoliation
KI	0.74	90
	1.47	0*
	2.94	0*
	7.35	0*
KBr	1.03	0
	2.05	0
	4.10	0
	10.25	0
KCl	1.65	0
	3.30	0
	6.60	0
	16.50	0
NaF	2.92	0
	5.83	0
	11.66	0
	29.15	0**

* Leaves severely desiccated and frozen on the stem.
** Slight marginal burning.

on bean plants. Only the iodide ion induced defoliation (table II). Desiccation and freezing which prevent defoliation were a result of excessive concentrations of the iodide ion and will be discussed below in more detail. The only other response to these halides was slight marginal burning at the highest concentration of NaF.

The application of the iodide ion was followed by an apparent desiccation of the leaf blades. This generally was visible 24 to 48 hours following application and increased in severity until the leaf abscised. The petiole also became yellowish with a sharp line appearing at the base of the pulvinus. The proximal side of this line appeared relatively normal.

The various oxidation states of iodine were examined (table III). All solutions were made up to contain iodine in amounts equivalent to that contained in KI when applied at the concentrations indicated. All were aqueous solutions with the exception of elemental iodine which was dissolved in a solvent system containing 2.5 % methanol, 2.5 % diethylether, 2.5 % glycerine, and 92.5 % water. The ability of I_2 to induce abscission supports the contention of a recent patent (8). The oxidation state of iodine

Table III

Influence of Iodine Oxidation State on Abscission of Tendergreen Bean Leaves

Iodine compound	% Defoliation							
	6 Days after treatment Iodide conc ($\times 10^{-2}$ M)				14 Days after treatment Iodide conc ($\times 10^{-2}$ M)			
	0.19	0.37	0.74	1.47	0.19	0.37	0.74	1.47
KI	0	100	61*	25*	6	100	72*	30*
I_2	0	61	88*	61*	61	100	100	100
KIO_3	61	44	39*	67*	78	61	39*	94*
H_5IO_6	6	11	28*	44*	22	61	61*	61*

* Remaining leaves severely desiccated and frozen on the stem (see text).

Table IV

Effect of Various Salts on Abscission of Primary Leaves of Tendergreen Beans

Iodide salt	% Defoliation							
	4 Days after treatment Iodide conc ($\times 10^{-2}$ M)				8 Days after treatment Iodide conc ($\times 10^{-2}$ M)			
	0.74	1.47	2.94	7.35	0.74	1.47	2.94	7.35
CdI_2	83	89	100	89	89	89	100	89*
ZnI_2	78	94	83	0	78	94	83	0*
NaI_2	72	83	88	11	100	100	72	17*
MgI_2	33	67	78	6	94	100	83	6*
KI	50	78	61	0	94	100	78	0*
NH_4I	22	50	39	0	94	89	50	0*
AlI_3	0	0	0	0	33	72	100	94*
CaI_2	17	28	39	0	50	72	39	17*
FeI_2	0	0	0	0	100	100	100	0*
$(CH_4)_4NI$	0	0	0	0	17	61	94	61*

* Leaves severely desiccated and frozen on the stem.

appears to influence the induction of premature abscission. The lower oxidation states are the most effective.

When various iodide salts were applied, it was found that the cation influenced the rate and degree of defoliation. Again, all solutions were made up to contain equivalent amounts of iodine based on the amount contained in the KI solutions. Table IV indicates the influence of the cation on the time and extent of response. Defoliation by aluminum, ferrous, and tetramethylammonium iodides was much slower than that caused by the potassium salt, although all of the compounds showed some abscission 8 days following treatment. The calcium cation caused a marked reduction in the percentage of defoliation both 4 and 8 days following application.

To determine if penetration through the roots followed by translocation through the stem would induce defoliation similar to that obtained with foliar treatment, solutions (50 ml) containing KI (200, 20, & 2 mg) were applied to the soil. Complete defoliation of primary leaves was observed at the 2 mg level. At the 20 and 200 mg/pot rate, desiccation and freezing due to excessive concentrations were observed. The results of foliar and soil applications are shown in figure 1.

When KI was applied to just one of the two primary leaves, it was observed that only the treated leaf abscised, whereas the opposite, untreated, leaf remained intact and showed no indications of injury. This result, with that of the soil application, indicate that the iodide ion is effectively transported upward, but that probably little or no translocation occurs downward out of the treated leaf or through the phloem.

Excessive concentrations of iodide ion caused leaf desiccation and freezing on the plant without defoliation (note, for example, the lack of abscission at higher concentrations in foliar and soil treatment, (fig 1). This effect was explored further by placing plants which had been excised at the soil level in beakers containing various concentrations of the iodide ion for either 3 or 24 hours. They were then removed, thoroughly washed, and placed in distilled water. Figure 2 shows the defoliation 8 days after treatment. Both durations of immersion indicate an optimum concentration of KI above which freezing of the leaves was observed. In the case of 3-hour immersion, the concentration was above 1,000 ppm; in the case of 24-hour immersion, it was above 125 ppm.

Table V

Effect of Decapitation on Abscission of Primary Leaves of Tendergreen Bean Plants Treated With KI*

Time of decapitation	% Defoliation Days after treatment		
	2	4	6
48 hr prior to treatment	11	100	100
24 hr prior to treatment	11	78	100
Day of treatment	6	50	100
24 hr after treatment	33	78	100
48 hr after treatment	50	100	100
Non-decapitated	56	100	100

* Iodide concentration 2.94×10^{-2} M

In those instances where excessive concentrations of the iodide ion resulted in desiccation followed by freezing, it was noted that the terminal meristem was killed. The possibility that an interaction existed between an active meristem and the point of abscission was examined. The terminal meristem was removed before, during, and after the application of KI (Iodide conc 2.94×10^{-2} M). Table V shows the reduction in defoliation when decapitation occurred at the time of treatment or within 4 days. Later decapitation was without effect.

In the preceding experiment, the single decapitation was followed by subsequent growth of axillary buds. These secondary buds apparently had replaced

Table VI

Effects of Removing Terminal & Secondary Buds on Abscission of Primary Leaves of Tendergreen Beans Induced by KI*

Treatment	% Defoliation Days after treatment		
	2	4	7
Decapitated & KI	0	11	11
Non-decapitated & KI	56	100	100
Decapitated control	0	0	0
Non-decapitated control	0	0	0

* Iodide concentration 2.94×10^{-2} M

Fig. 1. Effect of foliar and soil applications on KI on defoliation. *Left to right*: Untreated controls. Foliar application of 0.74, 1.74, 2.94, and 7.35×10^{-2} M. Iodide as KI application of 2, 20, and 200 mg of KI in 50 ml. of water.

Fig. 2. Effect of various iodide concentrations on excised bean plants immersed in solutions for 3 and 24 hours. *Extreme left*: Untreated control. *Upper row*: From left to right—62.5, 125, 250, 500, 1,000, and 2,000 ppm of KI for 3 hours. *Bottom row*: From left to right—concentrations as in the upper row immersed for 24 hours. Photo taken 8 days after beginning of the treatments.

Fig. 3. Effect of 2,3,5-triiodobenzoic acid (TIBA) on Tendergreen beans. *Left to right*—Untreated control, 2.48, 0.982, 0.491, 0.246, and 0.123×10^{-2} M TIBA.

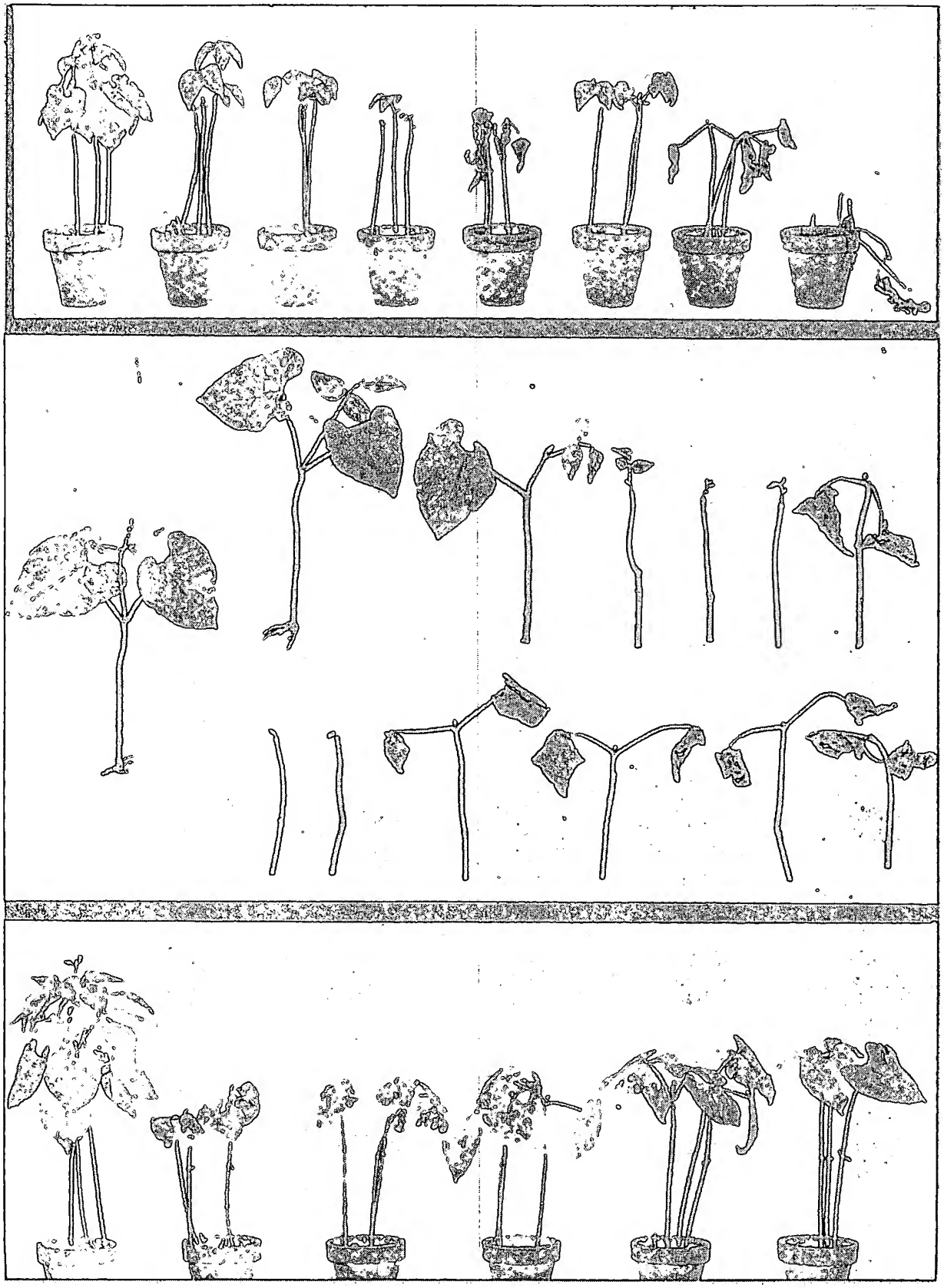

Table VII

Effect of Foliar Application of IAA Applied Simultaneously With KI to Tendergreen Beans*

Treatment	% Defoliation Days after treatment	
	3	6
KI & IAA (100 ppm)	0	17
KI & IAA (10 ppm)	44	44
KI	56	100
IAA (100 ppm)	0	0
IAA (10 ppm)	0	0

* Iodide concentration 2.94 × 10^{-2} M

the terminal meristem as growing points. To eliminate, as much as possible, all aerial meristemic growth, the primary terminal apex was cut off. The secondary buds were allowed to develop, then removed, and the plants were treated with KI. Table VI indicates nearly complete inhibition of defoliation when this procedure was followed. The normal desiccation associated with KI treatments was observed in the case of the decapitated plants, although abscission was nearly completely inhibited.

Previous workers have clearly established that indole-3-acetic acid (IAA) plays an important role in the control of abscission (1, 2, 3, 7, 11). Both promotive and inhibitory effects have been observed. The influence of IAA on iodide-induced defoliation was observed by application of the compound (10 & 100 ppm) as a foliar spray at the time of spraying the KI (iodide conc 2.94 × 10^{-2} M). It is apparent (table VII) that exogenous IAA inhibits the defoliation properties of the iodide ion when applied to the leaves only.

Table VIII

Effect of Photoperiod on Iodide-Induced Abscission

Iodide conc (× 10^{-2} M)	% Defoliation Days after treatment					
	3		5		7	
	Photoperiod		Photoperiod		Photoperiod	
	8 hr	16 hr	8 hr	16 hr	8 hr	16 hr
7.35	0**	0***	0	0	0	22
2.94	0***	50	5	88	15	100
1.47	83	55	100	88	100	100
0.74	83	0	100	72	100	77

** Complete death of the plants.
*** Nearly complete death of the plants.

Photoperiod is also known to influence abscission of plants (9). To determine if photoperiod affects the degree of defoliation observed with KI, Tendergreen beans were grown under 8- and the standard 16-hour photoperiods. The activity of the iodide was much greater in plants grown under 8-hour photoperiods than under 16-hour photoperiods (table VIII).

Susceptibility to iodide-induced abscission varies widely among different species and even between varieties of the same species (table IX). In other tests, cotton showed extremely high resistance to iodide-induced defoliation; approximately 1,000 times as much KI was required to defoliate immature cotton compared to the amount necessary to defoliate Tendergreen beans when applied to the soil. On the other hand, Coleus had approximately the same sensitivity as Tendergreen beans.

The stage of growth at the time of application will influence slightly the plant's degree of susceptibility

Table IX

Effect of KI on Abscission of Primary Leaves of Special Bean Varieties

Variety	% Defoliation Iodide conc (× 10^{-2} M)			
	7.35	2.94	1.47	0.74
Tendergreen	28*	39*	87	100
Black Valentine	11*	88*	100	89
Bountiful	11*	44*	87	89
French Horticulture	60*	44*	85	56
Pinto	0	0	0	0
Baby Lima	0	0	0	0

* Leaves severely desiccated and frozen on the stem.

to iodide-induced defoliation. Table X indicates this factor is not great, however. This also illustrates the iodide ion will induce abscission of trifoliate leaves as well as primary leaves.

Defoliation has been reported with 2,3,5-triiodobenzoic acid (TIBA) (12). A comparative test with KI and TIBA indicates the response induced by the latter compound (fig 3) was completely different from that observed with KI (fig 1). The iodide ion caused abscission of leaves present at the time of application leaving the new growth at the terminal apex relatively free from injury (unless excessive concentrations were used). TIBA, on the other hand, did not defoliate the primary leaves present at the time of application but caused abscission of the terminal apex and of all expanding trifoliate leaves.

Table X

Abscission of Leaves of Tendergreen Beans Treated at Various Stages of Growth With KI

Stage of development	% Defoliation Iodide conc (× 10^{-2} M)			
	5.65	2.26	1.13	0.57
10-day old, primary leaf expanded	100	100	89	78
15-day old, 1st trifoliate leaf expanded	44	100	100	...
35-day old, open flower	30	75	90	15
42-day old, mature fruit	0	70	90	80

Discussion

The iodide ion, either as an inorganic salt or organic complex, is the only halide which promotes abscission of Tendergreen bean leaves at the concentrations tested. This effect can be attributed to the iodide ion alone, although some effect of the cation was observed and is further substantiated by the ability of elemental iodine to induce abscission (8). The nature of this abscission is very different from that induced by TIBA, an organic iodine-containing compound.

That the cation exerts an influence on the rate and percentage of iodide-induced defoliation is evident. It is known that a deficiency of certain cations will induce premature leaf abscission. For example, suboptimal amounts of calcium, magnesium, and zinc induce premature abscission of citrus leaves (5). However, calcium was the only cation which reduced abscission induced by the iodide ion. There was no apparent pattern associated with the cation and further work will be required to clarify this point.

It is reasonable to suppose that transport of the iodide ion is by means of the xylem. This hypothesis is supported by observed movement of the ion from the roots to aerial portions of the plant. On the other hand, because the treated leaf of single leaf treatments was the only one to abscise, it is concluded that little or no movement occurred out of the treated leaf through the phloem. A concentration of the iodide ion sufficient to cause defoliation does not affect adversely the expanding trifoliate leaf. This may be considered to be due to the lack of phloem movement, or a consequence of biochemical retention of the iodide ion within the treated leaf.

Iodide appears able to induce leaf abscission rather generally among plants. Abscission has been induced in several different varieties of beans, Coleus (experiments not reported here show Coleus has approximately the same sensitivity to iodide-induced abscission as Tendergreen beans), and cotton. However, the degree of this response varies greatly. Immature cotton, for example, requires approximately 1,000 times as much of the iodide ion as the sensitive Tendergreen beans. The reason(s) for this difference is not known.

Although efforts were made to maintain uniform light intensities throughout, intensities under greenhouse conditions were variable. Lane and Hall (6) have shown light intensity to be important in chemical defoliation. The differences in the degree of susceptibility to iodide-induced defoliation (tables II & IV) could, in part, reflect differences in light intensity.

There are numerous indications of the role which IAA plays in leaf abscission. Probably the most widely accepted hypothesis is that of the auxin gradient (2) which requires a reduction in the amount of IAA in the leaf while the concentration in the stem remains relatively high. Although this work does not add any direct support, several points can be considered as indirect evidence for this hypothesis. The active meristem could be supplying necessary IAA to the stem. Elimination of this source of IAA either by physical decapitation or by rapid killing through excessive concentrations of the iodide ion, would account for the observed inhibition of abscission. The inhibitory influence of abscission by exogenously applied IAA to the leaves would also support this viewpoint. Hence, in the intact plant with an active meristem, it might be hypothesized that the iodide ion was inducing abscission by increasing, either directly or indirectly, the decomposition of IAA within the leaf, or by interfering in the normal biosynthesis of IAA. In either case there would be a net reduction of IAA within the leaf, as in the situation observed in bean leaves treated with the defoliant, NH_4SCN (10), while the IAA content in the stem was maintained. This would give rise to the auxin gradient hypothesized as necessary for leaf abscission. Studies to determine how the iodide ion induces abscission are currently in progress and will be the subject of subsequent communications.

Summary

The iodide ion is capable of inducing premature leaf abscission of intact plants. Although plants differ in their sensitivity to this phenomenon, it appears to be of general nature. An intimate relationship between iodide-induced abscission and IAA level also has been suggested.

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MONOFLUOROACETIC ACID AND RELATED COMPOUNDS[1]

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During and since World War II there has been a widening interest in the polyfluorinated hydrocarbons, most of which are very nearly inert biologically. On the other hand, a group of monofluorinated compounds has been found that includes many very toxic substances. Monofluoroacetic acid is the prototype of these compounds, which exhibit pharmacological actions of remarkably different character in different species. It is the purpose of this review to bring together and attempt to explain the available information concerning these potent pharmacological agents. The review has not been undertaken primarily because of interest in the fluoroacetates[2] *per se* but rather because their study contributes greatly to the pharmacological and biochemical understanding of drug actions.

Although first prepared synthetically by Swarts (122) in 1896, monofluoroacetic acid (FCH_2COOH) and its derivatives attracted very little attention from chemists and none from pharmacologists until the early 1940's when Polish chemists (88), escaping to England, brought word of the toxicity of the methyl ester of fluoroacetic acid which they had prepared (60). It was soon established by intensive studies under the auspices of the armed services in England and in this country that the fluoroacetates are exceedingly curious substances. The compounds are so toxic to dogs that 50 micrograms per kilogram cause prolonged convulsions of central nervous system origin and death from respiratory failure; yet they are only 1/200 as toxic to monkeys, in which species cardiac poisoning and ventricular fibrillation are the cause of death. The reasons for these seemingly capricious effects present problems of interest to biologists.

Security precautions during the war prevented prompt publication of research results and it therefore occasionally was difficult to acknowledge properly some of the investigative work done during that period. During this period Marais, in an independent study reported in 1944 (93), succeeded in isolating potassium monofluoroacetate from the South African plant, *Dichapetalum cymosum*. This plant, known locally as "Gifblaar," is a well-recognized hazard to stock and cattle (131). The occurrence of fluoroacetic acid in this plant is believed to be the first example of a naturally occurring organic fluoride; it completes the list of natural organic halogen compounds and presents an interesting problem in biosynthesis.

A considerable body of practical experience with the use of sodium fluoroace-

[1] Original investigations reported in this communication were supported (in part) by research grants from the Division of Research Grants and Fellowships of the National Institutes of Health, U. S. Public Health Service.

[2] The use of "fluoroacetates" as a loose generic name for this series of compounds is based on the fact that fluoroacetic acid and its simple compounds are the lowest active members of a homologous series.

tate (Compound # 1080, hence the common name of "ten eighty") as a rodenti-cide and general mammalian pest control agent has accumulated since Treichler and Ward of the United States Fish and Wildlife Agency introduced the compound for this purpose in 1945. The pertinent original literature should be consulted by those interested in this important application of fluoroacetate (5, 6, 66, 74, 92, 119, 128). It will be reviewed here only when information it contains contributes to the understanding of the biological actions of these compounds. The rodenticidal uses of the chemical cannot be ignored for such uses are of prime ecologic importance. The animal population of a poisoned area may be almost entirely destroyed by sodium fluoroacetate and its widespread use in pest control has been the cause of several human fatalities.

I. SPECIAL CHEMISTRY OF THE FLUOROACETATE COMPOUNDS

A. Synthesis

The synthesis of fluoroacetic acid, its derivatives and analogs has been studied intensively (60, 93, 122), particularly with the objective of perfecting large scale processes (7, 24, 72, 110, 111, 112, 113). Jenkins and Koehler have described the process and safeguards employed by the chief manufacturer of "1080", sodium fluoroacetate (73). Germane to the present review is the fact that most commercial procedures for production of "1080" result in some contamination of the product with fluorides (usually KF) unless special effort, usually redistillation of an ester of fluoroacetic acid, is taken to purify the product. This can be a source of error in interpreting the results of studies with fluoroacetate, particularly with high concentrations *in vitro*.

B. Structural Aspects

Certain details concerning the physical chemistry of fluoroacetate are important for an understanding of its pharmacological actions. The chemical behavior of monofluorinated organic acids in general is very little different from that of the corresponding unsubstituted acids; this is in contrast to the other monohalogenated acids. Indeed, the process of fluorination, with the corresponding shortening and strengthening of inter-atomic bonds, appears to confer increasing nonreactivity on all molecules (61).[3]

Attention has been directed particularly to the stability of the carbon-fluorine bond in fluoroacetic acid. There are little or no data upon the nature of this bond in longer-chain acids but their pharmacological behavior suggests that the stability of their C-F bond parallels that in fluoroacetate. It has been confirmed repeatedly that rupture of the C-F bond is very difficult (12, 13, 40, 110). Although some samples of sodium fluoroacetate do contain fluorides as contaminants, it is not reasonable to suppose that the fluoroacetates exert their actions by the liberation of fluoride, the pharmacological actions of which are quite different (108). Indeed, Bergmann and Fruton found that fluoroacetate, even after many hours in solution under physiological conditions of pH and temperature, loses no fluorine detectable as fluoride ion (13). However, boiling with 20 per cent KOH for 20 hours will release 50 per cent of the fluorine as KF (110). Bartlett and Barron have published a large list of biologically important chemical substances and enzymes with which fluoroacetate does *not* react, and have contrasted the four monohalogenated acetic acids with regard to their ability to thioacetylate cysteine (12) (see Table I). The rate of replacement of the fluorine of ethyl fluoroacetate by sulfite at 45° C. is expressed by a bimolecular velocity constant of 4.5×10^{-5}, whereas the bromine of ethyl bromoacetate is replaced at 25°C. at a very much greater rate (constant = 18.3), according to Backer and van Mels (8). This extreme nonreactivity of carbon-attached fluorine reflects the general relationship between the rates of reaction and the bond-energy values of the C-halogen bonds as well as the electro-negative values of the halo-

TABLE I

Physical and Chemical Properties of Acetic Acid and Monohalogenated Acetic Acids

(References to source of data are given in parentheses)

COMPOUND	DISSOCIATION CONSTANT OF ACID $K_a \times 10^{-5}$ (130)	ATOMIC RADIUS (104)	INTER-NUCLEAR DISTANCE IN ÅNGSTROMS (118)	BOND ENERGY IN KILO-CALORIES PER MOLE (103)	HALOGEN ELECTRONEGATIVITY (103)	REACTION RATE WITH CYSTEINE. TIME TO HALF REACTION AT 23°C. (12)	LD_{50} MICE Na SALT ORALLY
							mgm./kgm.
CH_3COOH.....	1.8	(H) 0.29	1.14	87.3	—	—	—
FCH_2COOH.....	210.	(F) 0.64	1.45	107.0 114.(59)	4.0	No reaction	17(125)
$ClCH_2COOH$.....	155.	(Cl) 0.99	1.74	66.5	3.0	125 mins.	165(100)
$BrCH_2COOH$.....	138.	(Br) 1.14	1.90	54.0	2.8	6.2 mins.	100(100)
ICH_2COOH.....	75.	(I) 1.33	2.12	45.5	2.5	4.0 mins.	63(100)

gens (Table I). It may be noted that, although chloroacetic acid is the strongest acid of the Cl, Br, I-monohalogenated acetic acids, iodoacetic is the most toxic and chloroacetic the least toxic. The toxicity is probably a reflection of their relative activity as thiol acetylating agents. But fluoroacetic acid, the strongest monohalogenated acetic acid, is also the most toxic. It is apparent that toxicity in this series is directly related to the reactivity of the halomethyl, rather than the carboxyl, moiety of the molecule. The great difference in chemical character between fluoroacetic acid and the more familiar iodoacetic acid is almost certainly a direct cause of the equally great difference in pharmacological action.

C. Analytical Aspects

There appears to be no chemical reaction given solely by fluoroacetic acid which would distinguish it from other compounds. Reactions of acetic acid which depend upon attack of the methyl group do not proceed at all or only under much more drastic conditions when attempted with fluoroacetic acid,

[3] The chemistry of the aliphatic fluorine compounds, as it was known in 1941, has been reviewed by Henne (61). Two additional recent reviews (14, 101) of the expanding field of fluorine chemistry should be consulted by those interested.

although there is no appreciable qualitative difference with regard to reactions involving the carboxyl groups. Fluoroacetate solutions give the characteristic blue color given by acetate and propionate with lanthanum salts (Kruger and Tschirch test). Hutchens and Kass (68) have made this test quantitative under very controlled conditions; for example, it is capable of detecting fluoroacetate in culture media in a range of 100 to 400 parts per million. The crystalline forms of the barium salts of the four monohalogenated acetic acids are useful for identification and have been described in detail (46). Characterization of sufficiently large amounts of pure fluoroacetic acid has been accomplished by the formation of conventional and suitable derivatives (60, 73, 110), especially fluoroacetamide (7), a useful intermediate.

Although there appears to be no great difficulty in characterizing fluoroacetic acid in the organic chemist's laboratory, quite the contrary is true when determination of small amounts of the compound in biological material is attempted. To date, nearly all methods depend upon splitting off and detecting the fluoride ion. Quantitative methods for the determination of fluoride with zirconium alizarin sulfonate (57) or as lead chlorofluoride (49) have been employed. Much has been accomplished toward the detection of fluoroacetate in drinking water by drastic measures to release the fluoride ion which are rather convenient for field use (57). Ramsey and Clifford have recently described (106) a method for the accurate and specific determination of fluoroacetate in food and other biological material in concentrations as low as 0.2 part per million, a degree of sensitivity which has very practical importance.

D. Stability of Sodium and Methyl Fluoroacetate Solutions

The extraordinary stability of the fluorine-carbon bond has attracted considerable attention and has led to the impression that compounds of fluoroacetic acid are very stable. This is not entirely true. Although solid sodium fluoroacetate, which is highly hygroscopic, keeps well in a desiccator, aqueous solutions of salt or esters decrease in toxicity with time. Albaum (2) showed that methyl fluoroacetate solutions decreased in toxicity even though refrigerated, while at room temperature the process was accelerated. Thus, a solution injected intraperitoneally in the calculated dose of 5 mgm./kgm. killed 7 of 10 rats when it was fresh but only 2 of 12 rats after it was kept 24 days in the refrigerator (approximately 5°C.). A second solution deteriorated at room temperature so markedly that, although 5 mgm./kgm. killed 19 of 24 rats when injected immediately after being prepared it killed but 8 of 20 rats after it had stood for only 7 days. Although precise assays have not been made, observations in this laboratory with solutions of sodium fluoroacetate and sodium γ-fluorocrotonate have indicated a similar rate of deterioration. As far as toxicity to yeast is concerned, however, fluoroacetate solutions remain unchanged for 1 month at 3° to 5°C. (77).

It is to be expected that hydrolysis of the methyl ester rapidly occurs and Price and Jackson (102) have found a half-life of less than an hour for this reaction at pH 7.0. This is not particularly important as a cause of the decreasing

tivity inasmuch as a similar decrease is noted with the sodium salt. As pointed out under section I-B, no fluoride ion is released under these conditions. Woods (133) has suggested the possibility that spontaneous decarboxylation accelerated by the resonance effects of the fluorine atom may occur according to the reaction

$$FCH_2COONa + H_2O \rightarrow NaHCO_3 + CH_3F\uparrow$$

with the liberation of the highly volatile, relatively non-toxic methyl fluoride. Thus, although no fluoride ion would accumulate in the solution, the actual content of fluoroacetate in the solution continually decreases. This type of reaction is often catalyzed by traces of halogen ions. Until more is known about this phenomenon, investigators using fluoroacetates should prepare solutions just prior to use. Deterioration does not seem to be a serious problem when solutions of sodium fluoroacetate are used in routine rat poisoning operations, perhaps because of the generally short (2 to 3 day) period of exposure of the poison (6).

II. TOXICITY

A. Response of Various Species to Toxic Monofluorinated Acids

Very few compounds are known which exert such variable pharmacological actions in different species as does fluoroacetate. Not only does the LD_{50} vary from 0.06 mgm./kgm. in the dog to well over 500 mgm./kgm. in the unique case of the South African clawed toad (*Xenopis laevis*), but the qualitative character of the pharmacodynamic action of the drug is equally varied. The major point of attack may be either the central nervous system or the heart. Both may be affected to varying degrees in some species, but it is usual to find that one organ is primarily concerned while the other is but slightly, or not at all, affected. Death may result from (a) respiratory arrest following severe convulsions, (b) gradual cardiac failure or ventricular fibrillation, or (c) progressive depression of the central nervous system with either respiratory or cardiac failure as the terminal event. All these responses follow a long and essentially irreducible latent period after the administration of the poison by any route. These phenomena are discussed in section III.

In Table II, the main toxic effect, if known, is indicated by 4+ and on the same scale 1+ indicates that this effect is very rarely seen. Although there is some difference between the sodium salt and the methyl ester of fluoroacetic acid when they are applied to frog tissue (18, 23, 38, 39), there appears to be no difference between them in the intact animal (30). No distinction between the two chemicals is made in Table II although most of the data were obtained with sodium fluoroacetate.

Study of the information presented in Table II indicates that, among the warm-blooded species, primates and all types of birds are generally the least susceptible to fluoroacetate poisoning, whereas the carnivora and *wild* rodents appear to be particularly sensitive. The Texan pocket gopher (*Geomys breviceps* sp.) is the most sensitive species so far described, all nine of those studied being killed by an intraperitoneal injection of 0.05 mgm./kgm. It is noteworthy that

TABLE II

Toxicity of Fluoroacetate Compounds

SPECIES	LD_{50}	ACCURACY[a]	ROUTE	HEART†	BRAIN†	REFERENCE
Sodium or methyl fluoroacetate; fluoroethanol						
	mgm./kgm.					
PRIMATES						
Man (*Homo sapiens*)	2–5	3	Oral	4+VF, F	2–3+C	6
Rhesus Monkey (*Macaca mulatta*)	4.0	2	I.V.	4+VF, F	2–3+C	30
Spider Monkey (*Ateles geoffroyi*)	15.0	2	I.V.	4+VF, F	0	30
UNGULATES AND RUMINANTS						
Goat	0.6	2	I.M.	4+VF	0	30
Sheep	2.0	3	Oral	4+VF	0	105
Horse	1.0	2	Oral I.M.	4+VF	1+C	30, 53
Swine (young)	0.4	1	I.P.	4+VF	4+C	30
Swine (adult)	<1.0	2	Oral	4+VF	4+C	115
CARNIVORES						
Canines:						
Dog (mixed breeds)	0.05	2	I.V.	0	4+C	30
	0.10(LD_{100})	1	I.V.	0	4+C	30, 37
Coyote (*Canis latrans nebracensis*)	0.10	1	I.P.	0	4+C	129
FELINES						
Cat (*Felis domesticus*)	0.20	1	I.V.	2+VF	4+C	99
RODENTS						
Rats:						
Albino Rats	5.0	1	I.M. S.C. I.P.	1+VF 2+F	3+C 4+D	30, 129
	2–3	2	S.C.	—	—	52
	2.5	—	Oral	—	—	74
Cotton Rat (Florida) (*Sigmodon hispidus littoralis*)	0.1	1	Oral	—	—	129
Wood Rat (California) (*Neotoma intermedia*)	1.5	2	Oral	—	—	129
Wood Rat (Arizona) (*Neotoma a. albegula*)	0.8	1	I.P.	—	—	129
Norway (Adult Wild Maryland) (*R. R. Norvegicus*)	0.22	1	Oral	—	—	44
Norway Rat (Florida)	3.0	2	Oral	—	—	129
Alexandrine Rat (*R. r. alexandricus*)	0.5	3	Oral	—	—	129
Black rat (*R. r. subsp.*)	0.1	—	Oral	—	—	74
Mice:						
Albino (Maple Grove)	19.3	1	S.C.	0	4+C	70
(Carworth)	17.0	1	Oral	—	—	125

TABLE II—*Continued*

SPECIES	LD_{50}	ACCURACY[a]	ROUTE	HEART†	BRAIN†	REFERENCE
Sodium or methyl fluoroacetate; fluoroethanol—*Continued*						
	mgm./kgm.					
Mice—Cont'd.						
Albino others	10.0	1	I.P.	—	—	129
	16.0	2	S.C.	—	—	105
	5.0	2	S.C.	—	—	52
Meadow Mouse (*Microtus (haydeni)*)	0.5	2	Oral	—	—	129
Deer Mouse (*Peromyscus sp.*)	4.0	1	Oral	—	—	129
House Mouse (*Mus musculus*)	8.0	2	Oral	—	—	129
Hamsters	3.0	2	I.P.	—	3+C 4+D	30
Rabbits:						
New Zealand White	0.25	1	I.V.	4+VF	0	30, 70
Pigmented	0.5	3	I.V.	4+VF	0	37
Dutch & others	0.5–1.0	3	S.C.	—		30, 70
Guinea Pigs	0.35	1	I.P.	0	4+C	30, 70
	0.25 (LD_{100})	2	S.C.	—	—	105
Ground Squirrels:						
Apache spotted (*Citellus spilosoma cavescens*)	0.4	3	I.P.	—	—	129
Columbian (*Citellus columbianus columbianus*)	0.9	2	I.P.	3+	4+D	129
Fisher (*Citellus b. Fisheri*)	0.3	—	Oral	—	4+C	129
Pocket Gopher:						
Breviceps—Texas (*Geomys breviceps sp*)	<0.05 (LD_{100} in 9 animals)		I.P.	—	—	129
Tuza—Florida (*Geomys floridanus*)	0.2	2	I.P.	—	—	129
Kangaroo Rats:						
Bannertail (*Dipodmys s. spectabilis*)	0.1	1	I.P.	—	—	129
Merriam (*Dipodomys m. merriami*)	0.15	2	I.P.	—	—	129
BIRDS						
Chickens:						
White Leghorn	7.5	1	Oral	—	3+D	42
Rhode Island Red	5.0	3	Oral I.V.	4+	3+D	30, 129
Plymouth Rock	5.5	2	Oral	—	—	129

TABLE II—*Continued*

SPECIES	LD_{50}	ACCURACY*	ROUTE	HEART†	BRAIN†	REFERENCE
	mgm./kgm.					
Pigeons:						
Florida	9.0	2	Oral	—	—	129
Colorado	2.5	2	Oral	—	—	129
Passerine:						
English Sparrow (*Passer domesticus*)	2.5	1	Oral	—	—	129
Game Birds:						
Gambels Quail (*Lophortyx gambeli*)	20	1	Oral	—	—	129
Carrion Feeding Birds:						
Golden Eagle (*Aquila chrysaetos*)	5.0	3	Oral	—	—	129
Black vulture (*Catharista urubu*)	15.0	3	Oral	—	—	129
POIKILIOTHERMS						
Frog:						
Rana pipiens	150.0	1	S.C.	0	2+C 4+D	30, 9
South African Clawed Toad (*Xenopis laevis*)	>500.0	2	I.P. S.C.	—	—	105
Sodium γ-fluorocrotonate $FCH_2CH{=}CHCOONa$						
Rhesus Monkey (*Macaca mulatta*)	2.5	3	I.V.	4+VF	2+C	37
Dog	0.05–0.07	2	I.V.	0	4+C	37
Rabbit (Albino)	0.15	2	I.V.	4+VF	0	37
Mouse (Albino) (*Rockland Swiss*)	1.0	2	I.V.	0	4+C	37
	2.0 (LD_{100})	1	I.V.	0	4+C	37
Rat (Albino)	1.0	2	I.P.	0	3+C 4+D	37
Rana pipiens	25.0	3	S.C.	—	2+C 4+D	37
Methyl γ-fluorobutyrate $F\text{-}CH_2CH_2CH_2COO\text{-}CH_3$						
Rhesus Monkey (*Macaca mulatta*)	3–5	3	I.V.	4+F	3+C	29
Cat	0.2	2	I.V.	0	4+C	29
Rabbit	0.10	1	I.V.	4+VF	1+C	29

TABLE II—*Concluded*

SPECIES	LD_{50}	ACCURACY*	ROUTE	HEART†	BRAIN†	REFERENCE
Ethyl 5-fluorohexanoate $F\text{-}CH_2CH_2CH_2CH_2CH_2COO\text{-}C_2H_5$						
	mgm./kgm.					
Rabbits	0.2–0.5	2	I.V.	—	—	109
Rats	2.3	2	I.M.	—	—	109
Mice	4.0	2	S.C.	—	—	109

Key to Scale: — = No information.
0 = Never seen.
1+ = Very rarely seen.
2+ = Occasionally seen. Can be expected.
3+ = Generally seen.
4+ = Characteristic action of drug. Always seen.

* Accuracy: 1—Highly accurate.
2—Accurate enough for practical work.
3—Estimate on few observations.

† VF = Ventricular fibrillation.
F = Cardiac failure (not VF).
C = Convulsion.
D = Depression.

‡ See (96, 109, 129) for data on a number of other species and compounds which were not suitable for inclusion in this table.

Laboratory strains of rats and mice are quite resistant to fluoroacetate, but that there is much variation between strains (see also section III-F, d). There is a tendency, to which the guinea pig is a striking exception, for herbivorous animals to manifest cardiac effects and for carnivores to develop *per primum* central nervous system convulsions or depression, whereas in more or less omnivorous species both the heart and central nervous system may be affected. Although a sex difference in respect to sensitivity has been reported for certain wild ducks (129), it has not been noted in other species. As might be expected, elevated environmental temperatures increase the sensitivity of mice to fluoroacetate to an appreciable degree (37).

Cold-blooded vertebrates are generally very insensitive to fluoroacetate, but it is clear that the increased toxicity of fluorocrotonate is much more apparent in *Rana pipiens* than in most mammalia. The sensitivity of frogs to fluoroacetate is not significantly increased when the frogs are kept in water at 32°C. (37), but frogs are more sensitive in the summer than in the winter (18).

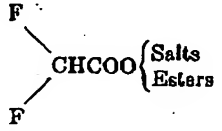
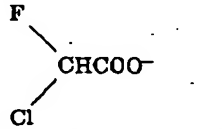
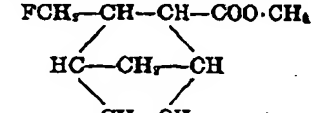
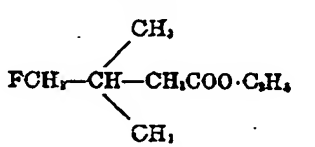
Such data as are available indicate that fish are relatively insensitive to fluoroacetate in the water surrounding them (81). *Anopheles larvae* are very sensitive to fluoroacetate (43). Indeed, the insects that have been studied (ants, roaches (5), aphids (65) and moths (72)) are generally very sensitive to fluoroacetate. Fleas are killed by feeding on poisoned rats (92). Microorganisms have not been studied extensively, but Kalnitsky and Barron (77) have made considerable use

of sensitive microorganisms and plant seedlings in elucidating the mechanism of action of fluoroacetate (v.i.). Mold growth (*Phycomyces Blakesleeanus* Morgan) is inhibited by fluoroacetate in low concentrations (1). As a matter of practical importance Gratch and coworkers (58) have reported that fluoroacetate in concentrations used for rat poisoning (approximately 2%) has no bacteriostatic properties against *P. pestis*. Therefore, it does not interfere with cultivation of these organisms from the tissues of rats obtained by poisoning operations with fluoroacetate.

B. Active and Inactive Fluorinated Compounds

As various monofluorinated compounds, almost exclusively aliphatic, were prepared and tested for toxicity, it became apparent that slight changes in structure were sufficient to abolish completely the dramatic fluoroacetate-like activity. In Table III the structures of a number of key compounds have been arranged under two columns: "active" and "inactive." (An "inactive" compound is one which has little pharmacological activity, or no more than might be expected from the corresponding non-fluorinated compound; for example, 50 mgm./kgm. of 1-fluoropropanol have no effect on rabbits (see footnote 3). Certain conclusions can immediately be made when such a tabulation is inspected. The only active compounds are straight-chain compounds with an *even number* of carbon atoms in which *one fluorine atom* is substituted in a *terminal position*. However, certain other requirements must be met. Thus, FCH_2CH_2Cl is inactive, although FCH_2CH_2OH is very active, presumably because little conversion of $-CH_2Cl$ to $-COOH$ can occur in the body, whereas fluoroethanol, analogously to unsubstituted ethanol, can be rapidly oxidized to the corresponding acetate in the body. Fixation of the α and β carbons of γ-fluorobutyrate in a methylene ring or loading the β carbon of γ-fluorobutyrate with one or two methyl groups results in inactivity. On the other hand, γ-fluoro β-hydroxy butyrates are very active. The conclusion is inescapable that compounds in the series which cannot form the fluoroacetate ion directly or through biochemical alteration have no characteristic fluoroacetate activity. This conclusion was understood in its essential features by McCombie and Saunders as early as 1943 (96).

It does not necessarily follow, however, that the toxicity of compounds capable of forming fluoroacetate *in vivo* is due *entirely* to the formation of fluoroacetate. There is evidence that γ-fluorobutyrate, for example, exerts a toxic action *per se*, independently of any action exerted by the fluoroacetic acid which may be formed by the β-oxidation of γ-fluorobutyric acid. Indeed, the toxicity and pharmacodynamics of the two compounds are quite dissimilar, which would appear to be sufficient evidence for a difference in mechanism. For example, progressive cardiac failure without ventricular fibrillation is noted in rhesus monkeys poisoned with fluorobutyrate, and fluorobutyrate-poisoned rabbits manifest signs of parasympathetic stimulation which are not characteristic of fluoroacetate (29). In addition, Kalnitsky and Barron have shown that rabbit kidney cortex, at least, does not convert fluorobutyrate to fluoroacetate and that the effects of the two agents are distinctly different (78).

Number of carbon atoms in longest straight fluorinated chain	Active	Inactive
1	None	$F\text{-}COO\text{-}C_2H_5$ $F\text{-}CH_3$
2	FCH_2COO {Salts / Esters}	 {Salts / Esters}
		
	FCH_2CHO	$FCH_2CH_2—O—CH_2CH_2COOH$
	FCH_2CH_2OH	FCH_2CH_2Cl
	FCH_2CN	
	FCH_2CONH_2	
	FCH_2COF	$ClCH_2COF$
	FCH_2COCl	$FCH_2CH_2SO_2Cl$
3	None	$FCH_2CH_2CH_2OH$ $CH_3CHFCOO\text{-}CH_3$
4	$FCH_2CH_2CH_2COO$ {Salts / Esters}	$CH_3CH_2CHFCOO\text{-}CH_3$
		
	$FCH_2CHOHCHCOO\text{-}CH_3$	
	$FCH_2CH=CHCOO$ {Salts / Esters}	
5	None	$FCH_2CH_2CH_2CH_2COO\text{-}R$
6	$FCH_2CH_2CH_2CH_2CH_2COO\text{-}R$	
7	—	—
8	$FCH_2(CH_2)_5COO\text{-}R$	—
9	—	—
10	$FCH_2(CH_2)_7COO\text{-}R$	—
11	None	$FCH_2(CH_2)_9COO\text{-}R$
12	$FCH_2(CH_2)_{10}COO\text{-}R$	

On the other hand, γ-fluorocrotonic acid does not appear to have any qualitative action in the intact animal different from that of fluoroacetate. This may be related to an increased ease of oxidation at the double bond, so that fluorocrotonic acid perhaps exists as such for a very much shorter time than does fluorobutyric acid. The greater toxicity of fluorocrotonate may be related to the greater ease with which it penetrates to the active area. Fluoroethyl fluoroacetate ($FCH_2COO \cdot CH_2CH_2F$) is about as active as fluoroacetate on an equimolar basis (111), but it acts much more quickly; this suggests an increased rate of cellular penetration (29).

Fluoroacetyl salicylic acid (110) may be cited as another example of a molecule which has an action not to be expected from the sum of actions of its groupings. It is as toxic to mice as fluoroacetate on a weight basis (compare acetylsalicylic acid); yet the characteristic action of fluoroacetyl salicylic acid is depressant rather than convulsant as would be expected if deacetylation with the liberation of fluoroacetate occurs in a manner similar to that described by Smith (120) for acetylsalicylic acid.

It appears that a fluorinated compound must have a structure capable of partially, but *not completely*, mimicking a natural metabolite if it is to be highly active pharmacologically. Factual evidence favoring this attractive interpretation of the data of Table III will be presented in Section IV. The serious consequences of such imperfect mimicry will be discussed in Section III.

III. RESPONSES OF ORGANISMS TO FLUOROACETATES

The majority of studies of a pharmacodynamic character have been made with methyl or sodium fluoroacetate. Some work has been done with fluoroethanol, but little beyond the determination of toxicity has been done with most of the other two-carbon analogs. There has not been a comparable amount of work with the four- and six-carbon compounds and it is possible, although not probable, that some modification of the conclusions concerning these compounds may be necessary in the future.

A. Absorption

The fluoroacetate compounds are all absorbed to some extent from all sites of application, although the lower members of the series are irregularly absorbed through the skin. Thus, application of methyl fluoroacetate in doses of 100 mgm./kgm. to the plucked skin of guinea pigs causes no poisoning according to Foss (52), although Saunders and Stacey (110) report an LD_{50} of 20 mgm./kgm. for methyl fluoroacetate placed on the clipped back of rabbits. The longer chain compounds and higher esters appear to be more readily absorbed; for example, 2-ethyl hexyl fluoroacetate on the shaved ear of a rabbit is lethal in 6 to 10 hours at a dose level of 10 mgm./kgm. (65). It is apparent that these compounds are not readily absorbed through the unbroken skin; but caution should be observed, especially when handling compounds likely to be oil-soluble.

Absorption through the pulmonary epithelium is very efficient in the case of the members of this series studied so far, and absorption of dusts of sodium fluoroacetate is equally effective (110). It is sufficient to point out that such data as are available indicate the same degree of toxicity for these compounds when they are inhaled as the esters as when they are injected as sodium salts. For example, Saunders and Stacey (110) have reported an LC_{50} in rabbits for methyl fluoroacetate of 0.1 mgm./l. (10-minute exposure). This corresponds roughly to their intravenous LD_{50} of 0.25 mgm./kgm.

There is no noteworthy difference between the toxicities of orally, subcutaneously, intramuscularly, intraperitoneally or intravenously administered methyl or sodium fluoroacetate (30, 93, 129). Very slight differences observed in the unexpected direction of an increased toxicity after oral administration (30) are probably not significant. Buckle, Pattison and Saunders (25) caution that methyl fluoroacetate is less toxic when injected subcutaneously in propylene glycol than in sodium chloride solution. Because of (1) solubility, (2) stability and (3) the long latent period before symptoms can be produced, which allows time for absorption, it may be concluded that there is no important difference between any non-percutaneous route of administration. This is an uncommon occurrence in pharmacology.

B. Distribution in Body Tissues

Knowledge of the distribution of fluoroacetate is largely inferential in origin. It might be assumed that the readily water-soluble sodium or methyl fluoroacetate would be distributed fairly evenly throughout body water. Recently, Ramsey and Clifford using their method for the determination of fluoroacetate, have presented data on orally poisoned rats (7 mgm./kgm.) which indicate an even distribution of fluoroacetate between the brain, heart, liver and kidney. Now that a method capable of detecting small amounts of fluoroacetate in animal tissues is available, it would be extremely desirable to ascertain whether the distribution of fluoroacetate is different in those species in which the heart or central nervous system is primarily affected. In this connection one experiment might be cited. Two dogs of approximately equal weight ate equal portions of heart muscle or skeletal muscle from a poisoned horse and were apparently capable of differentiating the amount of available fluoroacetate inasmuch as the dog eating the heart muscle died (53). It is common for domestic animals eating poisoned rats to be killed by the fluoroacetate still present in the rat (114).

C. Detoxication and Excretion

Observations that animals may be killed by the fluoroacetate remaining unaltered in poisoned animals or excreted unchanged in the urine (106) suggest that fluoroacetate is not changed in the body to any important extent. Urinary excretion is the only route so far demonstrated for the removal of the poison.

Although cumulation does occur (52, 105), it is not an outstanding characteristic of this poison. Administration of one half to one fifth of an LD_{100} daily will usually result in acute symptoms in 3 to 10 days; but if 3 days elapse between doses, repeated administration can be carried on indefinitely (52, 99). This suggests that elimination of that portion of the administered dose which was actually

exerting a toxic action is accomplished in about 24 hours. One may infer that at least some enzyme-fluoroacetate combinations are reversible.

D. Development of Tolerance

Tolerance to increasing doses of fluoroacetate has been demonstrated in the mouse and rat (35, 105, 127) and possibly exists in the rhesus monkey (37); but it could not be produced in the dog or rabbit (35, 37). Like cumulation, the development of tolerance is not a characteristic feature of the drug's action. The phenomenon is interesting, however, not only in its species specificity but also in its temporal and quantitative aspects. Rats receiving 0.5 mgm./kgm. (LD_{10}) by any route become largely resistant to the effects of 5.0 mgm./kgm. (LD_{75} for this strain) within more than 4 hours and less than 24 hours; this resistance lasts about 48 hours. It extends only partially to slightly higher challenging doses, and the ratio of doses cannot be extended; animals surviving 5 mgm./kgm. (with symptoms) are as sensitive as controls to 15 mgm./kgm. on the following day.

During the period of protection after 0.5 mgm./kgm., there is an increase in the ability of the rat to acetylate p-aminobenzoic acid. This may reflect partial inhibition of acetate turnover, resulting in the accumulation of a larger amount of acetate available for acetylation of foreign amines. Such an accumulation of acetate may also exert a protective action against a subsequent and larger dose of fluoroacetate (see sections III, IV).

E. Latent Period

All students of the actions of fluoroacetate have been impressed with the unusually long and variable latent period between the administration of the drug and the development of the characteristic response. This latent period occurs in all species so far studied, following intravenous injection. Application of concentrations of the methyl ester or the salts of fluoroacetic acid, equivalent to those existing in an intact animal poisoned with an LD_{50}, to the isolated heart and gut of the rabbit, the exposed brain of dogs, rabbits and monkey, or *in vivo* to enzyme preparations usually produces no immediate changes in the behavior of the organ or system. There is ordinarily no difference between these two agents except in the case of the isolated frog nerve preparation which Boyarski, Postel, Rosenblatt and Gerard (18) have found utterly refractory to the sodium salt but sensitive to the methyl ester. Although isolated intact frog muscle is more sensitive to the ester, the sodium salt is not inactive on this preparation (39).

To illustrate the latent period, data taken from current research in this laboratory may be used. An LD_{95} of sodium fluoroacetate (0.5 mgm./kgm.) injected intravenously in white rabbits requires 125 minutes (S.E. ± 12.7) to cause ventricular fibrillation and death. Symptoms of poisoning are not detectable for at least one-half hour after administration of the fluoroacetate. Increasing the dose to 25 or 250 mgm./kgm. will shorten the latent period to 20 minutes but cannot provoke the immediate responses characteristic of many drugs. Doses of the order of an LD_{20} may cause symptoms of poisoning and death of an occasional animal 48 hours or longer after administration.

The four-carbon compounds, γ-fluorocrotonate or the fluoroethyl ester of fluoroacetic acid, are more toxic than fluoroacetate and have a distinctly shorter latent period. For example, intravenous injection of an LD_{90-100} of sodium fluorocrotonate (0.3 mgm./kgm.) kills rabbits in about 60 minutes. Incomplete data indicate that dogs are very little more sensitive to fluorocrotonate in terms of the dose requirement but that the latent period for an LD_{100} (0.1 mgm./kgm.) is markedly shorter (approximately 90 minutes) than the 273 (S.E. ± 73) minutes for a corresponding LD_{100} of fluoroacetate (0.1 mgm./kgm.). Experiences of this nature suggest that the latent period can be shortened by increasing the chain length, thereby increasing the lipid solubility as well as decreasing dissociation, and thus facilitating cell penetration. No information is yet available on the latent period associated with the long chain compounds studied by Saunders (25, 109), although they are much more toxic than fluoroacetate. It has been shown (15) that the effectiveness of 2×10^{-6} M sodium fluoroacetate in decreasing the oxygen consumption of yeast is greatly increased by a low pH of the medium, a condition which increases the number of undissociated fluoroacetic acid molecules. This has also been found to be true of iodoacetic acid (3). In fact, undissociated molecules of acids are generally credited with being the form which actually penetrates cells (63).

The latent period in rabbits between the administration of sodium fluoroacetate and the onset of ventricular fibrillation or convulsions can be appreciably shortened by the prior administration of large amounts (approximately 1 gram/kgm.) of sodium bicarbonate, fumarate or chloride, but it cannot be eliminated entirely (36). The recent work of Hyde, Beckett and Gellhorn (71) has shown that certain agents facilitating cholinergic transmission potentiate many convulsant drugs. In mice, small asymptomatic doses of neostigmine (0.25 mgm./kgm.) greatly shorten the latent period of fluoroacetate (20 mgm./kgm.) induced convulsions, but do not eliminate it entirely. The decrease is to about one fifth of the control period (37). Neostigmine produced no change in the latent period when administered with fluoroacetate to dogs or rabbits. There does not seem to be any satisfactory explanation of these observations at present.

The latent period associated with fluoroacetates can probably be considered the result of at least two major factors: (1) the ability of the various fluorinated compounds to penetrate the cell, and (2) the time required for disruption of intracellular processes to become manifest as gross organ dysfunction; in principle this second factor is very similar to that which accounts for the latent period of pentylenetetrazol-induced convulsions. The future will decide which of the two factors is the more important.

F. Effect upon Intact Animals (30, 52, 53, 93, 105, 129)

The directly observable effects of an injection of sodium or methyl fluoroacetate, or fluoroethanol, in unanesthetized animals differ in many respects depending upon the species of animal employed (see Table II). They have been most frequently observed in rabbits, dogs, monkeys and rats, and these species seem to encompass the major variations in types of response.

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F. Effect upon Intact Animals (30, 52, 53, 93, 105, 129)

The directly observable effects of an injection of sodium or methyl fluoroacetate or fluoroethanol, in unanesthetized animals differ in many respects depending upon the species of animal employed (see Table II). They have been most frequently observed in rabbits, dogs, monkeys and rats, and these species seem to encompass the major variations in types of response.

: a) RABBIT (*Cardiac*): After an intravenous injection of sodium fluoroacetate (0.5 mgm./kgm.) in white rabbits, no change in the animal is discernible for about one-half hour. The first effect noted is usually a weakness of the neck and front legs and a decrease in activity. This state may progress to a marked extent but usually remains moderate until the occurrence of a sudden, violent convulsion of a clonic nature, typically associated with a cry. Opisthotonus, mydriasis and blanching of the retina rapidly develop, followed by progressive relaxation, a few gasping respirations and death. If the thorax be opened immediately, the auricles are found to be beating and the ventricles usually fibrillating. Occasional repeated convulsive bouts are always found to be the result of cardiac syncope, when the animals are carefully followed electrocardiographically (30). Fluorobutyrate causes considerable peristalsis and defecation in rabbits, a response not notable with the fluoroacetates. Except for the greater speed of action, fluorocrotonate is qualitatively indistinguishable from fluoroacetate.

b) DOGS (*Central nervous system*): The onset of fluoroacetate-induced effects (usually 4 to 5 hours after 0.1 mgm./kgm.) in the dog is heralded by a few minutes of barking and howling, "absence" (non-recognition of human presence), actions suggestive of fearful hallucinations, hyperactivity and finally a tonic spasm followed quickly by running movements. Tonic spasms and running movements may alternate or even completely cease, and the dog may appear normal at times; but ultimately the repeated anoxic assaults on the respiratory center during convulsions result in respiratory paralysis. The heart is often markedly slowed during convulsive seizures but rarely ceases activity until some time after the respiration has ceased. Death is typically the result of the effects of repeated and prolonged convulsions on the respiratory center, and never primarily cardiac in origin.

c) MAN AND RHESUS MONKEY (*Mixed response*): Although cats and pigs are perhaps more typical of the "mixed response" type of species (see 30), the rhesus monkey is of greater interest because such data as have been accumulated by the reviewer (through the most diverse channels, see also (52)) indicate that the response of adult man to fluoroacetate may be identical with that of the rhesus monkey. Children appear to be more prone to myocardial failure than to ventricular fibrillation as the terminal event; but, in general, they are very similar to the rhesus monkey in their responses to the poison.

As in man, in whom it may prove a diagnostic problem, the convulsive seizures due to fluoroacetate poisoning in the rhesus monkey are strikingly epileptiform. One or two hours after administration of the poison the animal may vomit and becomes apprehensive and seclusive. ("The first indication of poisoning in man is the onset of epileptiform convulsions after an initial period of nausea and mental apprehension" (52).) A few minutes later, actions suggestive of auditory hallucinations are followed immediately by nystagmus. Twitching of the facial muscles, often unilateral, heralds the onset of the convulsive seizure. It quickly spreads to involve the pinnae and the masseter muscles. Spread of the convulsive activity over the rest of the body is then very rapid, ending in a jerking, symmetrical convulsion in which the spasmodic, violent jerks may occur at a rate

...ter second. Tonic components are seen but do not dominate the pattern as they do in the dog. The animal is apparently unconscious during this period; but, as the seizure passes off, it will gradually attempt to regain its feet and ultimately does so about 30 minutes after the onset of the attack. The monkeys appear depressed for some time but often recover entirely from the convulsion. A complete second seizure is infrequently seen. Generally, the animal becomes weaker over the period of the next few hours (see cardiac status), but is often standing or otherwise exerting himself when suddenly stricken by ventricular fibrillation and death. Spontaneous recovery from ventricular fibrillation in the monkey is uncommon.

d) RATS (*Depression*): Although convulsions of a tonic nature, preceded by one- or two-hour period of decreasing activity associated with hypersensitivity to external stimuli, are the usual result of the injection of 5 mgm./kgm. of sodium fluoroacetate in rats of unspecified ancestry, death is the result of respiratory depression which gradually occurs long after convulsive activity has decreased or entirely ceased. Very large rats (over 400 grams) occasionally develop ventricular fibrillation; but, as might be expected from the general experience with fibrillation in small animals, this is uncommon. Some of the confusion in reports from different laboratories may be explained by a recent observation of the effects of the same dose of fluoroacetate in two strains of albino rats. Male Wistar rats received directly from the Wistar Institute convulse only occasionally and die after a period of respiratory depression lasting 5 to 24 hours. Comparable male rats of the Sprague-Dawley strain received directly from their stocks uniformly develop convulsions within 1 or 2 hours after injection and continue to convulse in a manner very similar to the dog. Death occurs in 4 or 5 hours (37). Although superficially these rats are identical, Anker (4) has demonstrated a very striking metabolic difference which will be discussed later. Rats which have survived an LD_{50} of fluoroacetate for 24 hours differ from most species (which are usually completely recovered in this time if they are to survive at all) in that they are still markedly affected. Disinclination to move is immediately apparent and is probably caused by a gross intention tremor which appears when the animal is forced to move. An extreme bradycardia can be detected by palpation or electrocardiographically (31). Complete recovery, if it is to occur, usually results within 48 to 72 hours after poisoning. Thiamine and atropine have no effect on these phenomena, but the tremor is temporarily quite exaggerated by diphenhydramine in doses that do not affect normal rats (37).

e) *Special responses in various species:* Ward and Spencer (129) noted emesis as a strikingly characteristic and early symptom of fluoroacetate poisoning in several species of carrion-feeding hawks and owls. Judged from the nature of the animals this is not surprising; nor does the profound sweating noted in poisoned horses by Frick and Boebel (53) appear unusual. Watt and Breyer (131) record that symptoms of poisoning in cattle which have eaten "Gifblaar" can be delayed by withholding water from the animal, a point of some theoretical and practical interest, and animals which recover are exceedingly thirsty. In this connection it is known that frogs allowed to imbibe water through their skin after

poisoning, which they do to the extent of a 50% weight gain, die more quickly than those kept dry (99). Mice are often anuric despite large amounts of parenteral fluids associated with therapeutic measures (70).

G. *Effect on Specific Systems*

a) *Cardiovascular:* The actions of fluoroacetate upon the cardiovascular system have been studied most extensively by Chenoweth and Gilman (39) who published a number of plates illustrating some of the phenomena described below. In general, most if not all the changes in the circulatory system produced by fluoroacetate can be explained by the action of the poison upon the heart itself; extracardiac effects, if present, are masked by the magnitude of the cardiac events. There is frequently a slight and transitory rise in mean arterial pressure which does not long persist. It is blocked by atropine and has been related by Foss (52) to the nicotine-like action of large doses of fluoroacetate. However, the general pattern is one of declining blood pressure (31, 105). Failure of the myocardial contractile power is steady and has been demonstrated in isolated heart-lung, perfused heart and papillary muscle preparations (31) as well as by direct inspection (105).

Constriction of the coronary arteries does not appear to occur and is certainly not the prime cause of the cardiac irregularities. During the course of poisoning no changes in capillary permeability to protein-bound dyes or in hemoconcentration, as indicated by hematocrit readings, have been observed (31). An elevation of hemoglobin levels in the goat has been ascribed to splenic contraction (52). As the heart decreases in contractile power it loses the ability to elevate the blood pressure in response to epinephrine or to compression of the descending aorta.

The development of numerous arrhythmias is apparent even on routine kymography, but the bizarre changes which occur can only be appreciated by electrocardiography, preferably by some technic permitting continuous visualization of the rapidly fluctuating changes. There appear to be differences even among cardiac-sensitive species in regard to the types of changes noted in the heart. Common to all, however, is a notable elevation in the amplitude of the T wave, although this perhaps most marked in the monkeys. Progressive downward shifting of the pacemaker was seen in the horse, goat (31) and sheep (105) and the electrical signs of activity in the auricle often disappeared. Prolongation of the P-R interval in the progressive fashion described by Wenckebach was especially marked in the goat, but was also seen in the cat. In direct opposition to this, the rhesus and spider monkeys showed no changes in auricular activity nor in auriculo-ventricular conduction.

Ventricular premature contractions are seen in nearly all species and are especially prominent in the rabbit and monkeys where they occur at first in a peculiarly systematic fashion (1:2, 1:4, etc.). Shifting alternation of the cycle length, QRS voltage, T wave height, shape, direction or take-off is very marked in the monkey and is often not predictably related to a *pulsus alternans*. The alternation of the pulse extends ultimately to a uniform 50% pulse deficit. This has also been reported in at least one fatal human poisoning. Ventricular fibrillation may occur at any time, but usually when the heterotopic ventricular arrhythmias are prominent; it appears to be initiated by mechanisms similar to those described by Wiggers for electrically-induced ventricular fibrillation. The occurrence of auricular fibrillation has not been noted in any species.

The actions of fluoroacetate upon the sensitive mammalian hearts are directed toward decreasing contractile power and disorganizing conduction and excitation. Failure or fibrillation as the end result is a manifestation of the relative importance of these effects in different species. It is not known that these effects are all the result of the same action of fluoroacetate, but it may be recalled that the specialized conduction tissue of the heart is contractile muscle tissue as well, suggesting that only a single action may be involved. There appears to be no relation between the gross amount of Purkinje tissue in the various species (56) and the development of conduction defects.

b) *Nervous system:* The magnitude of the direct effects of fluoroacetate upon the nervous system of sensitive species is apparent upon inspection of a poisoned animal. It must be emphasized that anoxic convulsions arising from cardiac syncope, such as ventricular fibrillation (in the rabbit, for instance), should not be confused with convulsions resulting from a *per primum* action of fluoroacetate upon the central nervous system, such as occurs in the dog. As indicated in Table II, both types of convulsions may occur in some species. This section will deal exclusively with seizures arising from a direct effect of fluoroacetate upon the nervous tissue. The pattern of these convulsions in intact animals has been described in section III F. With the exception of the remarkable similarity of fluoroacetate-induced convulsions in rhesus monkeys and in man to a *grand mal* epileptic seizure, the gross character of the convulsions in other species is not of great interest.

The regions of the central nervous system affected by fluoroacetate do not appear to be very sharply circumscribed. Studies on peripheral nerve have been carried out for the purpose of elucidating the mechanism of action of fluoroacetate, and will be discussed in section IV. The spinal cord can be shown to be sensitive: (a) by local application of fluoroacetate to the cord (34), with convulsive activity developing in a discrete area; (b) by the occurrence of convulsions below the level of a cord transection following an intravenous injection (34); and (c) by experiments in spinal and decerebrate cats (52). It usually requires supralethal doses to demonstrate involvement of the cord and it is probably of no importance in the intact animal. Curiously, spinal rhesus (?) monkeys twitch after 100 mgm./kgm. of methyl fluoroacetate but do not convulse (52). Reflexes mediated through the spinal cord of the cat are accentuated in the first few minutes of fluoroacetate-induced activity, but convulsions soon intervene and make further study impossible (52).

Recordings of the electrical activity of the brain, either directly from the brain surface or from the calvarium, of curarized or anesthetized animals have been made by Ward (126) and by Chenoweth and St. John (34). Using cats, Ward found that large doses of sodium fluoroacetate (2.0 mgm./kgm., 10 times the LD_{100}) injected intravenously or into the lateral ventricles produced marked

increases in electrical activity of subcortical areas, particularly the thalamus and hypothalamus. A high frequency, rather low amplitude activity was found to be particularly characteristic of the thalamus, while bursts of slow waves of a 3 to 8/sec. frequency in the cortex were noted which were synchronous with the envelope of the fast spikes of the thalamus. Slow wave activity of the hypothalamus was not regularly reflected in the cortex.

When smaller intravenous doses of fluoroacetate ($\frac{1}{2}$ to 2 times the LD_{100}) were administered to dogs under similar conditions, Chenoweth and St. John found an increased frequency and amplitude of waves recorded from temporo-parietal and occipital regions of the cortex but relatively little change in activity of frontal areas or cerebellum. Local application of fluoroacetate, either as the sodium salt or the methyl ester, resulted in relatively local changes in activity which spread so slowly as to suggest the probability that diffusion of the poison rather than primary radiation of the electrical activity was the cause of the increased involvement. They have stressed the apparent similarity of the spike and dome pattern often seen during the action of fluoroacetate to the electroencephalographic pattern of clinical *petit mal* epilepsy.

Electrical activity of the cortex reaches very high potentials during fluoroacetate poisoning but it can be obliterated by barbiturates and anticonvulsants (34) as well as by narcotic concentrations (9) of carbon dioxide (126). The sensitivity of rats to electrically-induced convulsions is increased about ten times by fluoroacetate (52). The apparent potentiation by neostigmine has been mentioned.

Rabbits poisoned by intravenous injections of fluoroacetate never reveal any electroencephalographic abnormalities until ventricular fibrillation occurs; but their brain tissue is not entirely resistant to fluoroacetate for convulsions typical for the dog can be induced in rabbits by the administration of sodium or methyl fluoroacetate directly into the cerebrum. This is, in a sense, a corollary to the observation that dogs prepared for electroencephalographic recordings under curare and artificial respiration occasionally develop ventricular fibrillation many hours after large doses of fluoroacetate, a fact indicating that the dog heart is not completely resistant to fluoroacetate (34).

c) *Other systems:* Because death from acute fluoroacetate poisoning is the result of cardiac or respiratory arrest in a relatively short and unpredictable period, there is little opportunity to observe changes in systems other than the heart or nervous system. The actions of fluoroacetate are probably exerted upon nearly all actively metabolizing tissues of the body, but the effects are difficult to demonstrate *in vivo*.

Skeletal muscle may be directly affected, notably in rabbits in which there is early head-drop and fore-limb weakness; these effects do not seem to be primarily the result of the lowered blood pressure, although the point has not been proven. Frog skeletal muscle is affected by fluoroacetate *in vitro* (38, 39). Contractions of isolated rabbit intestine are depressed by concentrations of sodium fluoroacetate of the magnitude presumed to exist in animals poisoned with an LD_{100} (50, 132).

Although kidney tissue is obviously very sensitive *in vitro* (see Section IV), renal dynamics do not seem to be much affected *in vivo*. Himwich *et al.* (62) noted an insignificant decrease in glucose Tm in chronically poisoned dogs, but accumulation of the poison occurred and convulsive death precluded the development of serious kidney malfunction. In 5 chronically poisoned cats (0.1 mgm./kgm. per day), with deaths at 3, 8, and 10 days and two survivors, high blood non-protein nitrogen levels of 134 and 120 mgm. % were noted in two cats at 8 and 10 days, respectively; this suggests definite renal damage (99).

Overall hepatic status is difficult to assess under any circumstances. A currently popular test of at least one function is the duration of anesthesia induced by short-acting barbiturates in laboratory animals. It has become apparent in recent studies in this laboratory that in fluoroacetate-poisoned animals there is a very great prolongation of the anesthetic effects of sodium thiopental, sodium pentobarbital, sodium phenobarbital and possibly even sodium barbital. The effect is not permanent, and the return to a normal duration of anesthesia is complete in less than a week. This has been observed in mice, rabbits, dogs and a rhesus monkey. It appears likely that it is the result of failure of the fluoroacetate-inhibited liver to detoxify these barbiturates, since the response to them is prolonged in proportion to the degree to which hepatic detoxication is believed to be important in their elimination. Studies on this and several related phenomena are currently in progress.

H. Pathological Changes

a) *Anatomical:* The histopathologic changes in fluoroacetate poisoning have not been described at length, perhaps because they do not contribute much to an understanding of its action. They appear to be largely the result of progressive cardiac failure with congestion of the abdominal viscera and lungs (52, 105). In the chicken there may be generalized petechial hemorrhages, especially noteworthy in lungs and ovaries (42), but this phenomenon appears quite specific for this species. Ordinary pathological studies do not seem to have been especially helpful in this field, nor are they likely to be so in the future.

b) *Biochemical:* Changes in the blood and tissue levels of various metabolites have been studied in a number of species of animals poisoned with fluoroacetate. A consistent increase in blood glucose levels, occasionally to 400 mgm./100 cc., has been reported in rabbits (94) and goats (52). Excretion of glucose in the urine may be expected to follow such a rise in blood sugar and does commonly occur in poisoned animals. Possibly some of the extra glucose in the blood is derived from liver glycogen since it has been found that poisoned rabbits have a marked reduction in liver glycogen (94). Lactic acid blood levels are elevated in poisoned rabbits (94, 95, 99), but it is exceedingly difficult to define the exact significance of such a change. Pyruvic acid blood levels rise and the lactate: pyruvate ratio is also increased (94). Preliminary experiments have indicated recently that blood and urine levels of acetic acid are elevated in dogs (37).

A considerable and rapid rise in serum inorganic phosphate has been reported in goats (52) and rabbits (95). Some of this phosphate may come from muscle